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13. ABSTRACT ***(Maximum 200 Words)***

The major objective of this research project was to identify and characterize promoter/enhancer elements that can be used to specifically target the expression of therapeutic genes to hypoxic regions within prostatic tumors *in vivo*. We have characterized sequences present within the human TNF-α promoter that are responsible for both constitutive activity and induction by hypoxia. We have produced a mutated version of this promoter that exhibits substantially reduced constitutive activity in epithelial cells while retaining responsiveness to various activating stimuli including hypoxia and ionizing radiation. While lacking prostate specificity, it is hoped nevertheless that this element may prove useful in various cancer gene therapy applications. In addition, we have cloned and characterized a large panel of novel sequences that exhibit high levels of constitutive promoter activity in prostate cell lines. Finally, we have identified a number of hypoxia inducible-promoter elements and are continuing to characterize these with respect to their prostate specificity.

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INTRODUCTION

The major objective of this research project was to identify and characterize promoter/enhancer elements that can be used to target the expression of therapeutic genes to hypoxic regions within prostatic tumors *in vivo*. Specifically, a novel episomal promoter/enhancer trap cloning system was developed and used to rapidly isolate candidate DNA sequences that were then tested both *in vitro* and *in vivo* for their functional activity in a variety of prostatic and other tumor cell lines under both normoxic and hypoxic conditions. Ultimately, adenoviral vectors in which expression of an indicator gene is driven off a candidate promoter/enhancer element will be constructed and tested for their ability to specifically target gene expression to hypoxic regions of prostatic tumor xenografts *in vivo*.

BODY/PROGRESS

Task 1: Isolation and characterization of novel "prostate-specific" hypoxia inducible promoter/enhancer elements

Initial efforts directed toward isolating and characterizing novel "prostate-specific" hypoxia-inducible promoter/enhancer elements for use in various cancer gene therapy applications utilized pEGTIII, a unique episomal promoter/enhancer expression vector that was developed in our laboratory. This vector contains a "leaderless" bone/liver/kidney alkaline phosphatase cDNA (ALP) that has been engineered to express a widely utilized splice acceptor site derived from CD44 exon v9 at its 5' end. Randomly generated size-selected (mean size around 2.5 kb) DNA fragments were cloned upstream of this indicator gene creating a plasmid library of moderate complexity (~10^7 recombinants). Upon transfection into an appropriate eukaryotic target cell, expression of ALP will only occur if the DNA fragment present in the plasmid vector contains both an appropriate promoter/enhancer element and an exon that can provide a splice donor site that can be utilized to generate an in-frame fusion protein. The vector also contains the EBNA-1 gene and an EBV origin of replication (oriP) and as such is expected to replicate episomally within the nucleus of transfected cells giving approximately 20-30 copies/cell. Such amplification is a potentially important aspect of the cloning strategy as it may allow the identification of promoter elements with weak constitutive activity that can then be excluded from further consideration potentially favoring the isolation of inducible promoters that are inactive in the absence of appropriate stimulation.

In initial "proof-of-principle" studies, the plasmid library was introduced into various prostatic (DU145, PC3, LnCap) and non-prostatic (293, T24, K562) tumor cell lines by electroporation. Forty-eight hours later, G418 was added at a final concentration of 200-400 μg/ml to select for transfected cells. Plates were then cultured for 18-21 days during which time discrete colonies containing 100-200 cells are produced. Colonies composed of cells transfected with a plasmid encoding a constitutively active promoter element could be readily identified by virtue of their expression of ALP. Briefly, plates were fixed for 1 minute in 100% ethanol then incubated for various periods of time with

the ALP substrate BCIP/INT. As shown in Figure 1, ALP-positive colonies stain an intense brown color and can be easily differentiated from ALP-negative colonies. The frequency of ALP-positive colonies (indicating the presence of constitutively active promoter elements) was, however, disappointingly low (~0.1%). Moreover, attempts to recover plasmid DNA from fixed ALP-positive colonies by scraping the cells from the dish and performing Hirt extraction were unsuccessful despite our considerable experience with the approach and the expenditure of much time and effort.

In an attempt to overcome this potentially serious obstacle to further progress we developed a novel colony lift technique that permits the identification of ALP positive colonies without the requirement that plates be first fixed in ethanol. Since the colonies remain viable, they can be picked and expanded for subsequent promoter isolation and characterization studies. Briefly, plates are washed extensively to remove medium then incubated in PBS for 20 minutes. Since PBS lacks calcium and magnesium ions the cells in the colonies round up and loosen their attachment to both the substrate and one another. At this point plates are overlaid with Immobilon-P PVDF membranes and incubated for 5 minutes at room temperature. A proportion of the cells present in each colony are bound by the membrane which is then removed, fixed in ethanol and stained for ALP expression using the ALP substrate BCIP/INT as before. Colonies that express ALP, presumably because they were transfected with a plasmid containing a promoter/enhancer element that is constitutively active in the cell type under study, can be readily identified by virtue of the presence on the membrane of a brown staining "plaque". The position of such colonies on the original plate can then be marked, fresh media added and the cells cultured for a further 48 hours to allow recovery. At this point, ALP-positive colonies can be picked and the clones expanded *in vitro* prior to further study. It was possible using this approach to establish a panel of cell lines that differ greatly in ALP expression, presumably reflecting differences in the activity of the constitutive promoter elements they contain. Examples of two such lines are shown in Figure 2. Although most of the colonies that were picked for further analysis in the course of this study were uniform in their expression of ALP and gave rise to cell lines that were essentially 100% ALP positive, occasional clones were observed in which only a small proportion of cells within the original tumor colony stained for ALP. Particularly common was a presentation where only the cells at the edge of a colony were strongly ALP positive. It is conceivable that the promoter element present within the pEGTIII plasmid with which these cells were transfected is active at only certain stages in the cell cycle or is regulated by signals associated cellular differentiation, activation, migration etc.

Unfortunately, despite exploring a large number of variations on the standard procedure that has served us well with respect to other episomal plasmids (e.g. pCDM8), attempts to isolate pEGTIII plasmid DNA from viable ALP-positive cells by Hirt extraction once again proved unsuccessful. In order to explain this finding, Southern blot analysis was carried out. These studies demonstrated unequivocally that in most of the established ALP-positive cell lines selected and maintained in G418, the pEGTIII vector was no longer episomal but rather, that one or more copies of the plasmid had instead integrated into the genome (data not shown). In those cell lines where episomal DNA

could be detected, copy number was generally very low (<5 copies/cell) and integration into the genome was usually also evident. Obviously, integrated plasmids will not be recovered by Hirt extraction and in those cells that do contain episomes copy number may be below the threshold required for efficient recovery of plasmids even when starting with a very large number of viable cells. Promoters could of course be isolated from ALP-positive cells by PCR using primers that flank the cloning site present in the pEGTIII vector. This was, however, not a particularly efficient process and is certainly not one that lends itself to high throughput screening. Indeed, for those cell lines in which only integrated plasmids are evident, a simple PCR approach employing primers that flank the pEGTIII multiple cloning site was rarely successful. This finding may reflect the fact that the recombination event that gives rise to stable integration may occur preferentially within the region of the plasmid that contains the genomic DNA insert.

Given the limitations of the pEGTIII approach discussed above, various alternative promoter-cloning strategies were explored. The most promising employed a plasmid vector designated pEpiNPT that was obtained from an industrial collaborator under a confidentiality agreement. As shown in Figure 3, this particular vector is similar in some respects to our pEGTIII plasmid as both are EBV-based and designed to replicate episomally within the nucleus of transduced cells. However, rather than using leaderless-ALP as an indicator gene, the pEpiNPT vector instead contains a cassette in which the neomycin resistance gene (NPT) is placed immediately downstream of a simple minimal promoter containing an appropriately positioned TATA box and transcriptional start site. A library is generated by cloning random genomic DNA fragments (mean insert size 3.5 kb) into a multiple cloning site adjacent to the minimal promoter. Since the minimal promoter itself lacks significant constitutive activity, resistance to G418 upon introduction of the library into an appropriate target cell line only occurs if the genomic DNA fragment present in a particular clone possesses the necessary complementing activity. The promoter elements isolated using this approach are thus artificial hybrids in that neither the minimal promoter or the corresponding genomic DNA fragment are expected to possess promoter activity in their own right but only when joined to one another. Initial screening indicated that a surprisingly high 10-12% of clones present within a library provided by our collaborator exhibited a level of promoter activity sufficient to confer resistance to G418 upon introduction into a range of prostatic and non-prostatic tumor cell lines. These results appeared very promising and encouraged further development of this alternative vector system. However, given the difficulty we had experienced previously in recovering plasmid DNA from cells transfected with the pEGTIII vector that had been selected and maintained for long periods of time in G418, it was felt necessary to develop an alternative transient screening strategy. Various approaches were explored but in the one that worked best, the NPT gene present in the pEpiNPT vector was removed and replaced with GFP allowing promoter activity to readily determined and quantified by FACS analysis. This vector was designated pEpiGFP. As shown in Figure 3, a second expression cassette was included in subsequent versions of this vector allowing transfected cells to be identified and/or selected. To permit cost effective high throughput analysis, a system was developed in which individual plasmid clones were isolated and introduced into

adherent tumor cells growing in the wells of a 96 well plate using either an 8 or 96 well electrode and two days later individual wells were harvested by trypsinization and any cells expressing GFP identified by FACS analysis. As illustrated in Tables I and II and Figure 4, considerable effort was expended in determining the most appropriate custom electrode design (i.e. conventional versus reverse polarity) as well as the optimal electroporation conditions necessary to obtain efficient gene transfer into a range of tumor cell lines. Although the efficiencies obtained were generally somewhat less than is seen for cuvette-based electroporations, a range of conditions were ultimately identified in which both the level of GFP expression and the percentage of GFP-positive cells obtained were deemed adequate to permit screening of the pEpiGFP library (Figure 4). Although labor intensive, this approach has lead to the identification of a number of promoter elements (~0.5% of the clones screened) that are constitutively active in prostatic and/or other tumor cell lines. Of these, a total of four elements were considered of particular interest as a result of their high activity and/or cellular specificity. As shown in Figures 6-8, these elements have been sequenced, their chromosomal locations defined and various potentially important structural elements identified. Details are provided in the corresponding Figure Legends. Particular interest was focused on promoter 780 by virtue of its differential activity in the prostate cell lines DU145 and PC3 (Table III). Initial deletional analysis of this promoter was carried out in order to narrow down the region responsible for its constitutive activity (Figure 9). Removal of the 5' end of the sequence (KpnΔ and XbaΔ) significantly enhanced activity suggesting the presence within this region of inhibitory/suppressive elements. Activity declined as addition 5' sequences were removed although even a 1103 bp Xho1 fragment that encompasses the 3' end of the promoter retained moderate activity. Of course even this relatively short sequence contains numerous putative transcription factor-binding sites as determined using the MatInspector v2.2 program accessed via the TRANSFAC Transcription Factor Database (http://transfac.gbf.de/TRANSFAC/). Ultimately additional deletional analysis will need to be carried out before site-directed mutagenesis can be used to identify the precise sequence elements responsible for the constitutive activity of this and other interesting promoter elements in prostatic tumor cells.

Both ourselves and the various collaborators to whom we have supplied reagents have begun the process of screening the pEpiGFP library using the same basic approach described above in order to identify promoter clones that are responsive to hypoxic stimuli. Since it was appreciated that the fluorescent activity of GFP is dependent upon the presence of O_2, cells were allowed to recover for various periods of time under normoxic conditions before analysis. Although several potentially promising prostate-specific and/or hypoxia-responsive promoter candidates were identified, to date none of these have withstood more rigorous analysis although screening studies are continuing.

Task 2: Characterization of the functional activity of candidate promoter/enhancer elements in prostatic tumor cells *in vitro* and *in vivo*

An important objective of this task was to characterize the functional activity of candidate hypoxia-inducible promoter elements in tumor spheroids that contain regions of diffusion-limited hypoxia. To this end we developed an approach in which highly reproducible tumor spheroids are established in hanging droplets in the wells of a Terasaki plate.

Since the promoter cloning work described above has not yet lead to the identification of novel elements that meet the stringent criteria for cellular specificity and responsiveness to hypoxia set out in the original proposal, initial functional studies focused instead on a 1399 bp genomic DNA fragment corresponding to a region of the human TNF-α promoter (position –1307 to +92) (Figure 10) that has been isolated, cloned into a modified version of the pEGTIII vector containing a full-length ALP cDNA (pEGTIV) and shown to possess both low constitutive activity and good induction following 18 hour incubation of transfected K562 cells (erythroleukemia) in a low O_2 environment. Unfortunately, in some prostatic and other epithelial cell lines, although significant induction was also obtained following exposure to hypoxia and/or other stimuli (see initial application), the TNF-α promoter exhibited high constitutive levels of activity that may limit its usefulness in targeted gene therapy applications (Table III). Initial stepwise deletional analysis determined that much of this constitutive activity could be attributed to a sequence located within a ~100 bp region immediately upstream of the TATA box (Figure 10). As shown in Figure 11, more targeted deletion of this short region while retaining the remainder of the promoter intact reduced constitutive activity by 80-90%. Individual transcription factor binding sites located within this region were then targeted using a PCR-based site-directed mutagenesis approach and in this way, the AP1 site located at position –108 was shown to play a critical role in the determining the constitutive activity of the promoter in non-myeloid cells. When placed upstream of a GFP indicator gene a "full length" TNF-αpromoter in which this site was rendered nonfunctional by site-directed mutagenesis exhibited minimal background activity. Using this construct as a starting point, additional deletional analysis is being carried out to define the sequence motif(s) responsible for the observed hypoxia-induced activity of the TNF-αpromoter. Particular emphasis is being placed on the three NF-κB sites shared by both the human and mouse TNF-α genes (Figure 10) as changes in redox potential resulting from irradiation or exposure to hypoxic conditions induce activation of NF-κB and tyrosine phosphorylation of its inhibitory subunit IkBα via a signal transduction pathway that involves Ras and Raf, but not MAP kinase. Moreover, recent studies have shown that interaction between NF-κB and the κ3 site located toward the 3' end of the promoter plays an important role in the transcriptional activation of TNF-α by superantigen. In contrast, studies with the monocytic cell line Mono Mac 6 indicate that LPS induces a factor with the characteristics of NF-κB that interacts with κ1, the most 5' of the three NF-κB binding sites in the promoter.

Task 3: Generation and analysis of adenoviral vectors in which expression of an indicator gene is driven off candidate hypoxia-inducible, prostate-specific promoter elements

Several adenoviral vectors have been generated in which expression of various indicator genes is driven off a number of the constitutive promoter elements examined in the course of the present study. Rather than using the approach outlined in the initial proposal, these vectors were constructed using the AdenoQuest Kit manufactured by QBio (Montreal), as the design of the "transfer" vector included in this kit facilitates the direct subcloning from pEGT or pEpi vectors of a cassette that includes the promoter of interest, an indicator or therapeutic gene and an SV40-derived polyadenylation signal. Initial studies have confirmed that, with the exception of PC3, which appears to express low levels of CAR, prostatic tumor cells can be efficiently transduced *in vitro* using such adenoviral vectors. Studies to evaluate the potential effectiveness of adenoviral-mediated gene transfer in the treatment of prostate cancer are currently being carried out by ourselves and various collaborators using a number of *in vivo* tumor models. The initial data from one such study is shown in Figure 12. In this experiment, DU145 tumor xenografts growing subcutaneously in SCID mice were directly injected with an adenoviral vector in which a constitutive promoter is used to drive expression of a chimeric gene encoding a cell surface protein consisting of the extracellular domain of the VEGF receptor Flk-1 fused in frame to the membrane spanning and cytoplasmic domain of the pro-apoptotic protein Fas. It is hypothesized that crosslinking of the Flk/Fas chimera by VEGF produced within the tumor microenvironment in response to hypoxia or other stimuli will trigger an apoptotic response in transduced cells. 24 hours after adenoviral administration, tumors were treated with photodynamic therapy (PDT) and the effect of adenoviral transduction on tumor regrowth determined. Even at the relatively low dose of virus used in these initial experiments some reduction in tumor regrowth is evident.

KEY RESEARCH ACCOMPLISHMENTS:

-Development of a novel high throughput screening strategy that can be used to rapidly isolate promoter elements that are constitutively active in a cell type of interest or responsive to a particular stimulus *in vitro*.

-Isolation and characterization of a panel of promoter elements that exhibit constitutive activity in prostatic tumor cells.

-Identification and preliminary characterization of promoter elements that exhibit differential activity in certain prostatic tumor cell lines.

-Identification of the transcription factor binding sites present within the human TNF-α promoter responsible for constitutive activity in transfected prostatic tumor cells.

-Generation of preliminary evidence that treatment with adenoviral vectors in which expression of a therapeutic gene (Flk/Fas) is driven off constitutive promoter elements can inhibit the growth of prostatic tumor xenografts *in vivo*.

REPORTABLE OUTCOMES:

Manuscripts will be submitted describing the characterization of the transcription factor binding sites present within the human TNF-α promoter that are responsible for constitutive activity and hypoxia responsiveness. The support of the US Army Medical Research and Materiel Command will be acknowledged.

The sequences of the novel promoter elements identified in this study will be submitted to GENBANK.

Aliquots of the pEGTIII and pEpiGFP libraries have been provided to colleagues in the US, Canada and the UK who are interested in identifying promoter elements that respond to a variety of stimuli including, hypoxia, radiation and tubulin-binding agents. We are actively involved in the planning and execution of these studies and will be included as authors on the resultant publications.

CONCLUSIONS:

Although the promoter cloning studies carried out to date have failed to identify a promoter element that is both prostate-specific and hypoxia-inducible, a high throughput screening strategy that will allow such sequences to be isolated has been developed and a number of potentially interesting constitutive promoter elements, some of which show differential activity in various prostate tumor cell lines, have been obtained. Screening studies are continuing and we are optimistic that therapeutically useful elements meeting the strict functional requirements outlined in the initial proposal will ultimately be identified and characterized using this approach.

REFERENCES:

N/A

APPENDICES:

Figures 1-12 and Tables I-III attached in Appendix 1.

FIGURE 1

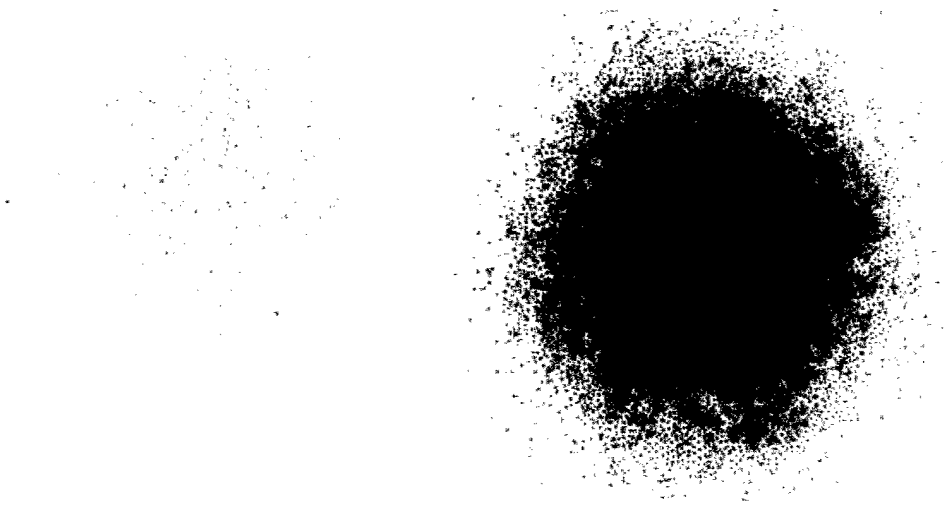


Figure 1: Identification of promoter elements active in prostatic tumor cell lines. The pEGTIII plasmid library was introduced into various prostatic (DU145, PC3, LnCap) and non-prostatic (293, T24, K562) tumor cell lines by electroporation. Forty-eight hours later, G418 was added at a final concentration of 200-400 μg/ml and the plates cultured for 18-21 days to select for transfected cells which gave rise to discrete colonies containing 100-200 cells. To determine ALP expression, plates were fixed for 1 minute in 100% ethanol then incubated for various periods of time with the ALP substrate BCIP/INT. As shown in the right hand panel above, colonies that are ALP-positive (presumably because the plasmid with which they were transfected contained a constitutively active promoter) can be readily detected by virtue of their intense brown staining and can be easily differentiated from ALP-negative colonies, an example of which is shown in the left hand panel. Irrespective of the cell line tested, the frequency of ALP-positive colonies produced was around ~0.1%.

FIGURE 2

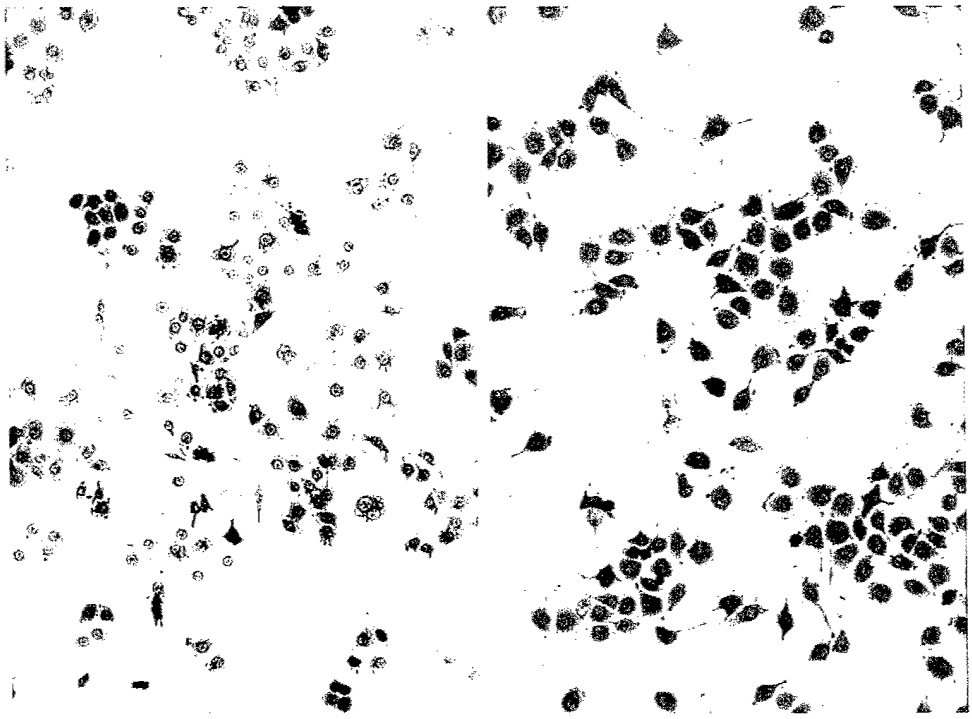


Figure 2: Variations in ALP expression by established cell lines derived from pEGTIII transfected ALP-positive tumor cell colonies. ALP-positive tumor cell colonies identified using the colony lift technique described in the text, were picked and expanded *in vitro*. As illustrated by the two T24 clones shown above, the populations obtained differ greatly in their expression of ALP as determined by BCIP/INT staining of ethanol fixed cells adherent to glass coverslips. Such heterogeneity can be attributed at least in part to differences in the activity of the particular constitutive promoter element that is present within the pEGTIII plasmids with which the cells were initially transfected.

FIGURE 3

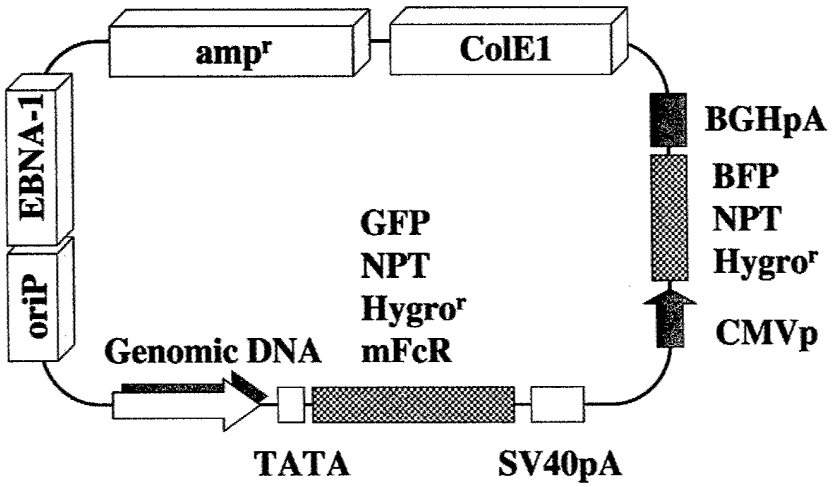


Figure 3: Key features of the pEpi promoter cloning vector series

TABLE I: Determining optimal electroporation conditions for "conventional" 8/96 well electrode.

Sample #	µF	%viable	$x+\sigma^{n-1}$	%GFP +ve	$x+\sigma^{n-1}$	x FL-1	$x+\sigma^{n-1}$
Data 001	NIL	97.72		0.08		N/A	
Data 002	NIL	98.00	97.7+0.2	0.06	<0.1	N/A	N/A
Data 003	NIL	97.52		0.02		N/A	
Data 004	50	39.72		5.69		424	
Data 005	50	56.72	49.8+8.9	10.17	8.8+2.7	269	328+84
Data 006	50	52.82		10.49		290	
Data 007	100	49.57		9.62		312	
Data 008	100	47.04	48.7+1.4	11.82	10.6+1.1	314	312+2
Data 009	100	49.48		10.43		311	
Data 010	200	48.50		12.45		541	
Data 011	200	41.64	45.7+3.6	15.05	14.0+1.4	470	516+40
Data 012	200	47.10		14.52		536	
Data 013	400	38.88		17.57		444	
Data 014	400	43.16	39.8+3.0	20.25	19.0+1.4	549	514+61
Data 015	400	37.38		19.21		549	
Data 016	800	15.08		6.5		248	
Data 017	800	39.94	31.6+14.3	20.47	14.3+7.1	596	440+177
Data 018	800	39.62		15.96		475	

TABLE II: Determining optimal electroporation conditions for "reverse-polarity" 8/96 well electrode.

Sample #	μF	%viable	$x+\sigma^{n-1}$	%GFP +ve	$x+\sigma^{n-1}$	x FL-1	$x+\sigma^{n-1}$
Data 019	NIL	96.66		0.00		N/A	
Data 020	NIL	96.58	96.0+0.1	0.00	<0.1	N/A	N/A
Data 021	NIL	94.90		0.00		N/A	
Data 022	50	24.32		1.32		49	
Data 023	50	16.00	22.74+6.1	0.50	0.9+0.4	55	53+3
Data 024	50	27.90		1.00		54	
Data 025	100	55.68		13.25		546	
Data 026	100	46.96	51.1+4.4	13.81	12.9+1.1	400	476+73
Data 027	100	50.78		11.59		482	
Data 028	200	53.90		14.99		375	
Data 029	200	53.0	54.1+1.2	18.39	16.2+1.9	397	431+78
Data 030	200	55.35		15.36		520	
Data 031	400	36.19		17.10		247	
Data 032	400	40.44	37.5+2.6	21.19	20.9+3.7	635	551+272
Data 033	400	35.79		24.43		771	
Data 034	800	24.45		8.10		301	
Data 035	800	25.11	24.4+0.8	6.67	9.2+3.2	165	287+116
Data 036	800	23.53		12.78		395	

FIGURE 4

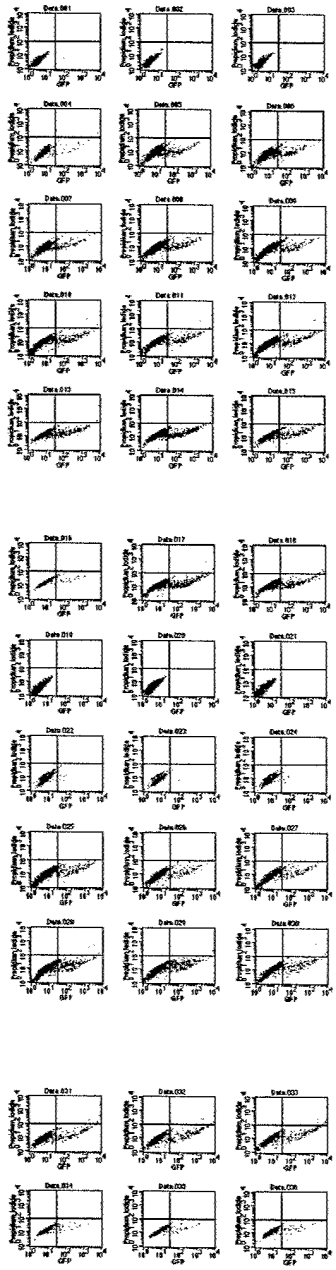
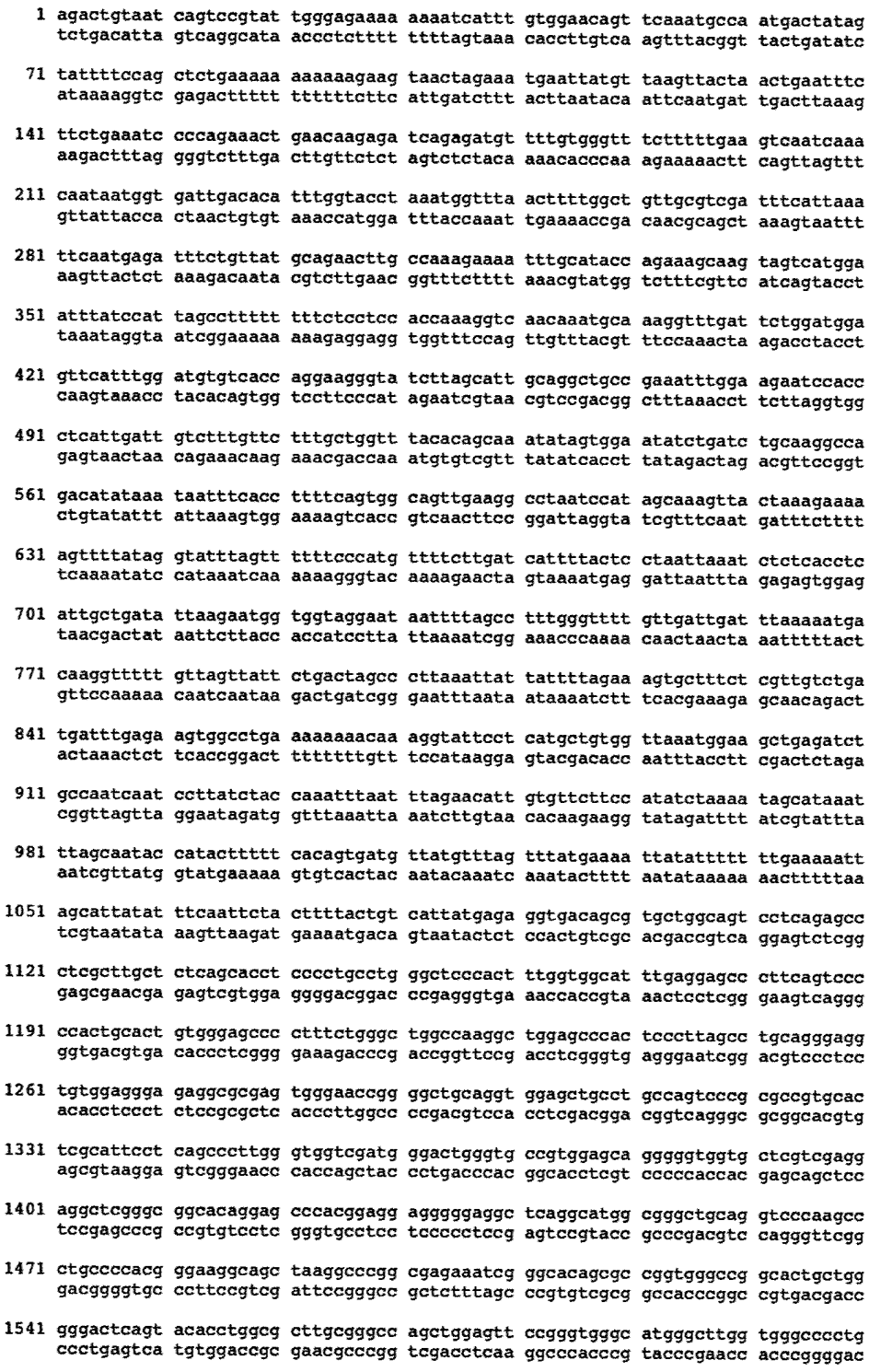


Figure 4: Determination of optimal conditions for electroporation of tumor cells in flat-bottomed 96 well plates. 10 ml of a 293 cell suspension (1 x 10^5 cells/ml) were added to 100 ml pEpiGFP(CMV) plasmid DNA (1 mg/ml), mixed well and 100 μl aliquots added to each well of a flat-bottomed 96 well plate (1 x10^4 cell/well). Wells were electroporated at 280v, R3 (48 Ohms) at capacitance

settings of 50, 100, 200, 400 and 800 μF using a BTX electroporator and the time constants were recorded (see Tables I & II). Immediately after electroporation, 100 ml DMEM+20% FCS were added to each well and the plates incubated at 37°C, 5% CO_2 for 3 days. To determine GFP expression, any non-adherent cells were collected, wells trypsinized and the adherent and non-adherent populations pooled. Cells were pelleted by centrifugation at 1000 rpm for 10 min, resuspended in 750 μl. HBSS+2% FCS containing 1 μg/ml propidium iodide (PI) and analyzed on a FACSCalibur. Non-viable PI-positive cells were gated out and data collected on the percentage viable (PI-negative) cells, the percentage of viable cells expressing GFP and the mean fluorescence of GFP-positive viable cells. The dot plots obtained are attached and the data is summarized in Tables I & II. Data 001-018: Conventional electrode. Data 019-036: Reverse polarity electrode.

FIGURE 5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561 gacatataaa taatttcacc ttttcagtgg cagttgaagg cctaatccat agcaaagtta ctaaagaaaa
ctgtatattt attaaagtgg aaaagtcacc gtcaacttcc ggattaggta tcgtttcaat gatttctttt
631 agttttatag gtatttagtt ttttcccatg ttttcttgat cattttactc ctaattaaat ctctcacctc
tcaaaatatc cataaatcaa aaaagggtac aaaagaacta gtaaaatgag gattaattta gagagtggag
701 attgctgata ttaagaatgg tggtaggaat aattttagcc tttgggtttt gttgattgat ttaaaaatga
taacgactat aattcttacc accatcctta ttaaaatcgg aaacccaaaa caactaacta aatttttact
771 caaggttttt gttagttatt ctgactagcc cttaaattat tattttagaa agtgctttct cgttgtctga
gttccaaaaa caatcaataa gactgatcgg gaatttaata ataaaatctt tcacgaaaga gcaacagact
841 tgatttgaga agtggcctga aaaaaaacaa aggtattcct catgctgtgg ttaaatggaa gctgagatct
actaaactct tcaccggact ttttttgtt tccataagga gtacgacacc aatttacctt cgactctaga
911 gccaatcaat ccttatctac caaatttaat ttagaacatt gtgttcttcc atatctaaaa tagcataaat
cggttagtta ggaatagatg gtttaaatta aatcttgtaa cacaagaagg tatagatttt atcgtattta
981 ttagcaatac catacttttt cacagtgatg ttatgtttag tttatgaaaa ttatattttt ttgaaaaatt
aatcgttatg gtatgaaaaa gtgtcactac aatacaaatc aaatactttt aatataaaaa aacttttaa
1051 agcattatat ttcaattcta cttttactgt cattatgaga ggtgacagcg tgctggcagt cctcagagcc
tcgtaatata aagttaagat gaaaatgaca gtaatactct ccactgtcgc acgaccgtca ggagtctcgg
1121 ctcgcttgct ctcagcacct cccctgcctg ggctcccact ttggtggcat ttgaggagcc cttcagtccc
gagcgaacga gagtcgtgga ggggacggac ccgagggtga aaccaccgta aactcctcgg gaagtcaggg
1191 ccactgcact gtgggagccc ctttctgggc tggccaaggc tggagcccac tcccttagcc tgcagggagg
ggtgacgtga cacctcgggg gaaagacccg accggttccg acctcgggtg agggaatcgg acgtccctcc
1261 tgtggaggga gaggcgcgag tgggaaccgg ggctgcaggt ggagctgcct gccagtcccg cgccgtgcac
acacctccct ctccgcgctc acccttggcc ccgacgtcca cctcgacgga cggtcagggc gcggcacgtg
1331 tcgcattcct cagcccttgg gtggtcgatg ggactgggtg ccgtggagca gggggtggtg ctcgtcgagg
agcgtaagga gtcgggaacc caccagctac cctgacccac ggcacctcgt cccccaccac gagcagctcc
1401 aggctcgggc ggcacaggag cccacggagg agggggaggc tcaggcatgg cgggctgcag gtcccaagcc
tccgagcccg ccgtgtcctc gggtgcctcc tccccctccg agtccgtacc gcccgacgtc cagggttcgg
1471 ctgccccacg ggaaggcagc taaggcccgg cgagaaatcg ggcacagcgc cggtgggccg gcactgctgg
gacggggtgc ccttccgtcg attccgggcc gctctttagc ccgtgtcgcg gccacccggc cgtgacgacc
1541 gggactcagt acacctggcg cttgcgggcc agctggagtt ccgggtgggc atgggcttgg tgggcccctg
ccctgagtca tgtggaccgc gaacgcccgg tcgacctcaa ggcccacccg tacccgaacc acccggggac

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1611 cactcggagc agccagccag ccctgctggc cccgggcaat gggggactta gcacctgggc cagtggctgt
     gtgagcctcg tcggtcggtc gggacgaccg gggcccgtta ccccctgaat cgtggacccg gtcaccgaca

1681 ggagggtgta ctgagtcccc cagcagtgcc ggcccaccgg cgctgtgccc gatttctcgc cgggccttag
     cctcccacat gactcagggg gtcgtcacgg ccgggtggcc gcgacacggg ctaaagagcg gcccggaatc

1751 ctgccttccc gtggggcagg gcttgggacc tgcagcccgc catgcctgag cctccccctc ctccgtgggc
     gacggaaggg caccccgtcc cgaaccctgg acgtcgggcg gtacggactc ggaggggggag gaggcacccg

1821 tcctgtgccg cccgagcctc ctcgacgagc accaccccct gctccacggc acccagtccc atcgaccacc
     aggacacggc gggctcggag gagctgctcg tggtggggga cgaggtgccg tgggtcaggg tagctggtgg

1891 caagggctga ggaatgcgag tgcacggcgc gggactggca ggcagctcca cctgcagccc cggtgtggga
     gttcccgact ccttacgctc acgtgccgcg ccctgaccgt ccgtcgaggt ggacgtcggg gccacaccct

1961 tccactgggt gaagccagct gggctcctga gtctggtggg aatgtggaga gtctctatat ctagctcagg
     aggtgaccca cttcggtcga cccgaggact cagaccaccc ttacacctct cagagatata gatcgagtcc

2031 gattgtaaat acaccaatca gcaccctgtg tttagctcaa ggtttgtgag tgcaccaatt gacactctgt
     ctaacattta tgtggttagt cgtgggacac aaatcgagtt ccaaacactc acgtggttaa ctgtgagaca

2101 atctagctgc tctggtgggg ccttggagaa cctgtgtgtc tagctcaggg attgtaaata caccaatcgg
     tagatcgacg agaccacccc ggaacctctt ggacacacag atcgagtccc taacatttat gtggttagcc

2171 cactctgtat ctagctcaac gtttgtaaac acaccaatca gcaccctgtg tttagctcaa ggtttgtgag
     gtgagacata gatcgagttg caaacatttg tgtggttagt cgtgggacac aaatcgagtt ccaaacactc

2241 tgcaccaatc gacactctgt atctagctgc tctggtgagg atgtggagag tctttatatc tagctcaggg
     acgtggttag ctgtgagaca tagatcgacg agaccactcc tacacctctc agaaatatag atcgagtccc

2311 attgtaaaca caccaatcag caccctgtgt ttagctcaag gtttgtgagt gcaccaatcg acactgtatc
     taacatttgt gtggttagtc gtgggacaca aatcgagttc caaacactca cgtggttagc tgtgacatag

2381 tagctgctct ggtgaggaca tggagaacct ttatgtctag ctcaaggatt gtaaatacac caatcggcac
     atcgacgaga ccactcctgt acctcttgga aatacagatc gagttcctaa catttatgtg gttagccgtg

2451 tctgtatcta gctcaaggtt tgtaaacaca ccaatcagca ccctgtgttt agctcaaggt ttgtgagtgc
     agacatagat cgagttccaa acatttgtgt ggttagtcgt gggacacaaa tcgagttcca aacactcacg

2521 accaatcgac actctgtatc tagctgctct ggtggggcct tggagaacct gtgtgtggaa actctgtatc
     tggttagctg tgagacatag atcgacgaga ccaccccgga acctcttgga cacacacctt tgagacatag

2591 taactaatct gatggggacg tggagaacct ttgtgtctag ctcagggatt gtaaacgcac caatcagcgc
     attgattaga ctacccctgc acctcttgga aacacagatc gagtccctaa catttgcgtg gttagtcgcg

2661 cctgacaaaa caggccactc ggctctacca atcagcagga tgtgggtggg gccagataag agaataaaag
     ggactgtttt gtccggtgag ccgagatggt tagtcgtcct acacccaccc cggtctattc tcttattttc

2731 caggctgccg gaaccagcat tggcaaccca ctcgagtccc cttccacctc gtggaa
     gtccgacggc cttggtcgta accgttgggt gagctcaggg gaaggtggag cacctt
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Figure 5: Complete sequence of pEpiGFP promoter clone #648. Maps to human Chromosome 6. Matches nucleotides 10084-12869 of Genebank Accession gi|9581538|emb|AL135912.7|[9581538]. Potentially important features noted within this region of chromosome 6 include a CpG island at position 11358-12004, an endogenous retroviral LTR12 element at position 12618-13250 and three pTR5 repetitive elements (an endogenous retroviral sequence that is present in human but not baboon DNA) at positions 11169-11398 (matches 743-963 of consensus sequence), 11372-11638 (matches 1074-1345 of consensus sequence) and 11609-12610 (matches 926-1907 of consensus sequence).

FIGURE 6

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1    aagcttgcct ccttctcccc gcagtggcca ggacaaggct cacatttttc tgttctctgt ctccccagcc
     ttcgaacgga ggaagagggg cgtcaccggt cctgttccga gtgtaaaaag acaagagaca gaggggtcgg

  71 ctctcctggg ctttctcccg gtgcaatgac accttcatct ccctgttggt cccattctct ggggtggctg
     gagaggaccc gaaagagggc cacgttactg tggaagtaga gggacaacca gggtaagaga ccccaccgac

 141 gaggtggcct gggtatgctc ggtccagtgc aggtgggcgt gcggactctt ccgtacctca cttctccctg
     ctccaccgga cccatacgag ccaggtcacg tccacccgca cgcctgagaa ggcatggagt gaagagggac

 211 cacctgtggc ccctgctatc ggcgtgctta ctggagatgc gattcccctc ttccttcttt gttcaggtag
     gtggacaccg gggacgatag ccgcacgaat gacctctacg ctaaggggag aaggaagaaa caagtccatc

 281 cagggcccaa gaaaaatcgt cttttatgga aggcatcaag caagaaattt gataggcatt ctcttcactc
     gtcccgggtt ctttttagca gaaaatacct tccgtagttc gttctttaaa ctatccgtaa gagaagtgag

 351 tagaaatcag ccccgtggga caggggtctt gcccctttatt ctctgacagc ggccagctcc tagggcacgc
     atctttagtc ggggcaccct gtccccagaa cgggaaataa gagactgtcg ccggtcgagg atcccgtgcg

 421 ttggtgcgtg gggacactca acagggagat gctttctctg tgtcaataga cttcactcat ggactcagtc
     aaccacgcac ccctgtgagt tgtccctcta cgaaagagac acagttatct gaagtgagta cctgagtcag

 491 gttcgcctaa aaatagattc caaatggttg gaaactcata ggacagtggt ttggcacaaa agcgtgcttg
     caagcggatt tttatctaag gtttaccaac ctttgagtat cctgtcacca aaccgtgttt tcgcacgaac

 561 tggctggttt taaaactaag tcgcgaaatt gatacactgc tgtgtgatac accagaaagt ggtccgttcc
     accgaccaaa attttgattc agcgctttaa ctatgtgacg acacactatg tggtctttca ccaggcaagg

 631 atccacccgc gcaagtgtct gtcacacaga ggttagttct ttcataaaac tttattacgt atcgaacccc
     taggtgggcg cgttcacaga cagtgtgtct ccaatcaaga aagtattttg aaataatgca tagcttgggg

 701 attactcatc ctgaatctgt ttcaggaagg atgtgtgctg gtgaagagga tgagcgaagg cctgccttcc
     taatgagtag gacttagaca aagtccttcc tacacacgac cacttctcct actcgcttcc ggacggaagg

 771 tgaagctcga gcgctcatag ggaagatgtt ccgtaaacaa agaaacacgc atggagcatg tcatgtcccc
     acttcgagct cgcgagtatc ccttctacaa ggcatttgtt tctttgtgcg tacctcgtac agtacagggg

 841 atgtggagag agccacggag cacagggggc cgggaggggc tggatgcgcc agtggccagc ttggggaagg
     tacacctctc tcggtgcctc gtgtcccccg gccctccccg acctacgcgg tcaccggtcg aaccccttcc

 911 cctctcttgg ggcttcctcc tttggctcct ggagtctaaa acttcctggt gtcccttctg cctgcctagc
     ggagagaacc ccgaaggagg aaaccgagga cctcagattt tgaaggacca cagggaagac ggacggatcg

 981 agaacccctc cacacggctc cccgtctaag gcggcaggga tgagctgtgt ggatgctgct gagactggtg
     tcttggggag gtgtgccgag gggcagattc cgccgtccct actcgacaca cctacgacga ctctgaccac

1051 tgactgcttt gagccttgtc cactgatgca gcacctgcag ggtgccgccg gccccagcaa ggacacacca
     actgacgaaa ctcggaacag gtgactacgt cgtggacgtc ccacggcggc cggggtcgtt cctgtgtggt

1121 gtggttggtg ggatgtgagc gggactggag tgaggccagc cgtggacagc agtgcagagg gacaggccct
     caccaaccac cctacactcg ccctgacctc actccggtcg gcacctgtcg tcacgtctcc ctgtccggga

1191 agggagttgt gctttaaaga agggcaggag tcaaatgtcc agggtggagg tgtgcggcca agggagagtt
     tccctcaaca cgaaatttct tcccgtcctc agtttacagg tcccacctcc acacgccggt tccctctcaa

1261 gtgttaaaaa tgtgagatga cagggccggg cgtggtggct catgcctata atcccagcac tttgggaggc
     cacaattttt acactctact gtcccggccc gcaccaccga gtacggatat tagggtcgtg aaaccctccg

1331 caaggcggat ggatcatgag gtcaagagat cgagaccatc ctggccaaca tgttgaaacc cgtctctact
     gttccgccta cctagtactc cagttctcta gctctggtag gaccggttgt acaactttgg gcagagatga

1401 aaaaatacga aaattagctg ggcgtggtgc aggagaatct cttgaaccca ggaggcagag gtggcagtga
     tttttatgct tttaatcgac ccgcaccacg tcctcttaga gaacttgggt cctccgtctc caccgtcact

1471 gccgagatca cgccactgca ctacagcctg gtgacagagc aagacttcgt cacacacacg cacaaaaaag
     cggctctagt gcggtgacgt gatgtcggac cactgtctcg ttctgaagca gtgtgtgtgc gtgttttttc

1541 tgagatgaca gctcatgttt agtgccgggt gctgggtgag cgagggtggg tgagcatcca tttcccaggg
     actctactgt cgagtacaaa tcacggccca cgacccactc gctcccaccc actcgtaggt aaagggtccc
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1611 gaggggcccc gcgcccatgg agactgctgg ggacaggctg gggcagggcg gggtggaggg tgtcctcttc
     ctccccgggg cgcgggtacc tctgacgacc cctgtccgac cccgtcccgc cccacctccc acaggagaag

1681 tgatggatgc
     actacctacg
```

Figure 6: Complete sequence of pEpiGFP promoter clone #764. Maps to human Chromosome 2. Matches nucleotides 102210-103899 of Genebank Accession gi|14717362|gb|AC079400.6|[14717362]. Potentially important features noted within this region of chromosome 2 include an Alu repeat at position 102210-103899.

FIGURE 7

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   1 caagctttgc tatatctgaa actcaaggtt gtctctgcta aatttcagta aacaattgtg aaagtcatga
     gttcgaaacg atatagactt tgagttccaa cagagacgat ttaaagtcat ttgttaacac tttcagtact

  71 tgatggataa tcactgtcgt ctctacaggt ccactatcct atgcctcaat atgctgacct ttatgttctt
     actacctatt agtgacagca gagatgtcca ggtgatagga tacggagtta tacgactgga aatacaagaa

 141 tgatttcctg ttaggtttgg ccaatgggaa gcaacagcaa gagatggata gatggaagga gagagagctg
     actaaaggac aatccaaacc ggttaccctt cgttgtcgtt ctctacctat ctaccttcct ctctctcgac

 211 gagaatttct ttccttacat cagttgggta gctgggcaga ttgactgggt ctgaaaccaa acacaggttc
     ctcttaaaga aaggaatgta gtcaacccat cgacccgtct aactgaccca gactttggtt tgtgtccaag

 281 agctacttgc cacttgcaag gtcaaaaatc aaggacaagg tggggtggaa ggaaggaaag gaatcgccaa
     tcgatgaacg gtgaacgttc cagtttttag ttcctgttcc accccacctt ccttcctttc cttagcggtt

 351 ggctttgtgc ctgaaaggaa ccatttcaaa tttctggata gaacgcaagg gcttaaaaag ggaggttggt
     ccgaaacacg gactttcctt ggtaaagttt aaagacctat cttgcgttcc cgaatttttc cctccaacca

 421 ggtgggaggg gcatgcaaga ggggcaagga ggtgccagtc agtctatgta agttgctctg atgacttgag
     ccaccctccc cgtacgttct ccccgttcct ccacggtcag tcagatacat tcaacgagac tactgaactc

 491 ttattacccc atctgttgaa cgggctggca ccatcccagg cacaatcggg gtgaaagcta actgcagctt
     aataatgggg tagacaactt gcccgaccgt ggtagggtcc gtgttagccc cactttcgat tgacgtcgaa

 561 tgagtaatct cctggaggag gagaattcca taggtaccta gattgtttca agactcagtc catgaacttc
     actcattaga ggacctcctc ctcttaaggt atccatggat ctaacaaagt tctgagtcag gtacttgaag

 631 caagaaaaca tataattaga tgagggggac attgtgcaag ggagtgcctg gtggaaggaa agagagcaaa
     gttcttttgt atattaatct actccccctg taacacgttc cctcacggac caccttcctt tctctcgttt

 701 ggttaccata taattttcaa gcatctaaac ataaagtaag caaggaggaa aatggaaaaa gattaaaaaa
     ccaatggtat attaaaagtt cgtagatttg tatttcattc gttcctcctt ttacctttt ctaatttttt

 771 ttaaaaatag agtatttggt tacatgtcct tgaccctctg ctgaaggcca cagcttatga ccaccctctc
     aatttttatc tcataaacca atgtacagga actgggagac gacttccggt gtcgaatact ggtgggagag

 841 ctacagctac cagagtctcg gtttccagta actcgttgtt ccttcctctg gctcatcagg cccaggaatg
     gatgtcgatg gtctcagagc caaaggtcat tgagcaacaa ggaaggagac cgagtagtcc gggtccttac

 911 atagcagctt cctgttattg ctagtcatgg ggtgtttcat tatctcttgt tatttttcct taactctact
     tatcgtcgaa ggacaataac gatcagtacc ccacaaagta atagagaaca ataaaaagga attgagatga

 981 cactcctttg taaacagacc tttcactaat aactcttcac tctttccatc tgctttcagc cagaaccctg
     gtgaggaaac atttgtctgg aaagtgatta ttgagaagtg agaaaggtag acgaaagtcg gtcttgggac

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1191 accgctggta accatcattt tgttctttgt ctttgtgtct tcgacatttt tttttttta aagattacac
     tggcgaccat tggtagtaaa acaagaaaca gaaacacaga agctgtaaaa aaaaaaaaat ttctaatgtg

1261 atttaagtga gatcatgcaa tatttttctt tctgtgtctg gagttttgca gtttcaggtc ttacatttaa
     taaattcact ctagtacgtt ataaaaagaa agacacagac ctcaaaacgt caaagtccag aatgtaaatt

1331 gcctttaatc cattctgagc tgattttgtg tgtggcataa gaaaagaatc ctattttata ttttattata
     cggaaattag gtaagactcg actaaaacac acaccgtatt cttttcttag gataaaatat aaaataatat

1401 tttcatttgg tttttaattt ttaatttcat caactgtgac tttgttgtat gaaaagagtg tgggtagcaa
     aaagtaaacc aaaaattaaa aattaaagta gttgacactg aaacaacata cttttctcac acccatcgtt

1471 atctcttgct ccactttgga aatcccctcc cctcccacta aagtggagat tcctacctaa tctaactcct
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1541 ggagggttgt taacagagat taaaagagac agtgaagtgg acatgcttta aaataaaaat gtgttttata
     cctcccaaca attgtctcta attttctctg tcacttcacc tgtacgaaat tttatttta cacaaaatat
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```
1611 agagaggcag cataatctgg agactcagag cacaggcacc agaggcaaca ctaggtttga agctccagct
     tctctccgtc gtattagacc tctgagtctc gtgtccgtgg tctccgttgt gatccaaact tcgaggtcga

1681 ctgcctctga taaacttata atttaaatct gtgcctcagt ttcctcatat gtaaaatgag gataataaca
     gacggagact atttgaatat taaatttaga cacggagtca aaggagtata cattttactc ctattattgt

1751 gatgtacctc ttagggttgt tgtcggagtt aaatgaaata taatattgcc tgatatatag taagttctca
     ctacatggag aatcccaaca acagcctcaa tttactttat attataacgg actatatatc attcaagagt

1821 gtcagtggta cttttctttt ttttttttt ttttatgaga cagagtcttg ctctgttgcc caggctggag
     cagtcaccat gaaaagaaaa aaaaaaaaaa aaaatactct gtctcagaac gagacaacgg gtccgacctc

1891 tgcagtggcg tgatctcggc tcaatgcaag ctctgactcc caggttcaca ccactctcct gcctcagcct
     acgtcaccgc actagagccg agttacgttc gagactgagg gtccaagtgt ggtgagagga cggagtcgga

1961 cctgagtagc tgggactaca ggcgcccgcc accacacccg gctaactttt tttgtatttt tagtagagat
     ggactcatcg accctgatgt ccgcgggcgg tggtgtgggc cgattgaaaa aaacataaaa atcatctcta

2031 ggggtttcac cctgttagcc agggtggtct cgatctcctg acctcgtgat ccacccacct cggcctccca
     ccccaaagtg ggacaatcgg tcccaccaga gctagaggac tggagcacta ggtgggtgga gccggagggt

2101 aagtgctggg attgagaggt gacaatgtgc tagcagccct tgcttgctct cggcacctcc tcaggccaca
     ttcacgaccc taactctcca ctgttacacg atcgtcggga acgaacgaga gccgtggagg agtccggtgt

2171 gcgtccactc tggctatgct cgaggagccc ttcagcccgc cactgcactg tgggggcccc tctctgggct
     cgcaggtgag accgatacga gctcctcggg aagtcgggcg gtgacgtgac acccccgggg agagacccga

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2311 gatgggcgtg gtgctcgtgg gctagtgcga gttccaggtg ggcgggggct tggcggccca cacttggagt
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2381 ggctggctgg catcgccggc ctgcacagtg aggggtttag cacccgggcc agcagctgcg gaaggtgtgc
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     ccaaggggtt gtcgacggcg gggggcgagg tgccgtgggt cagagtagtt gtcgggttcc cgactcctca

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     cgtccgtccc gcaccctgac cgcccgtcga ggcgggtgcc cggatcacac cctaggtgat ccttttcagt

2731 gctgggctct tgagtcgggt ggggacttgg agaaatttta tgtctagctg gaggattcta tatgcaccaa
     cgacccgaga actcagccca cccctgaacc tctttaaaat acagatcgac ctcctaagat atacgtggtt

2801 tcagcactct gtgtctagct cagggtttgt ggatgcacca atcagcactc tgtatctagc taatctggtg
     agtcgtgaga cacagatcga gtcccaaaca cctacgtggt tagtcgtgag acatagatcg attagaccac

2871 gggacttgga gaacttttat gtctagctag aggattgtaa atgcaccaat cagcactctg tgtctagcta
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2941 aaagattgta aacacgccaa tcagcattct gtgtcaggct caaggtttgt aaacacacca atcagtgctc
     tttctaacat ttgtgcggtt agtcgtaaga cacagtccga gttccaaaca tttgtgtggt tagtcacgag

3011 tctgtctagc taatctagtg gggacttgga gaacttttac atctagctag agtattgtaa atacaccaat
     agacagatcg attagatcac ccctgaacct cttgaaaatg tagatcgatc tcataacatt tatgtggtta

3081 cagcactctg catctagctc agggattgta aatgcaccaa tcagcgccct gtcaaaacgg accaatcagc
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3151 tctctgtaaa atggaccaat cagctctttg taaaatggac caatcagcag gatgtgggtg gggtcagata
     agagacattt tacctggtta gtcgagaaac attttacctg gttagtcgtc ctacacccac cccagtctat

3221 agggaataaa agcaagctgc ctgggtcagc agcggcaacc cgcttgggtc ccctttcacg ctgtggaagc tt
     tcccttattt tcgttcgacg gacccagtcg tcgccgttgg gcgaacccag gggaaagtgc gacaccttcg aa
```

Figure 7: Complete sequence of pEpiGFP promoter clone #780. Maps to human Chromosome 5. Matches nucleotides 158043-154752 of Genebank Accession gi|18449986|gb|AC093240.2|[18449986]. Potentially important features noted within this region of chromosome 2 are discussed below.

FIGURE 8

```
   1 aagcttacat tctagtgagg atgttagctt tgggggaaga ggctgtacca agcacatgcc tggaacaggg
     ttcgaatgta agatcactcc tacaatcgaa acccccttct ccgacatggt tcgtgtacgg accttgtccc

  71 aaggagctta aaaatatctg tggaatgagt gactaaatgc ccagaatgaa tcagaggtga cactgcaaat
     ttcctcgaat ttttatagac accttactca ctgatttacg ggtcttactt agtctccact gtgacgttta

 141 gttcagctgg tgggtaaaaa agaatgactt gcttaaccca ttccctgcca tgctccatcc catcctcctt
     caagtcgacc acccatttttt tcttactgaa cgaattgggt aagggacggt acgaggtagg gtaggaggaa

 211 tcctgcccag agggccctcc tgctgtggtc caaggggcag ctggctgtgc agagagcagg cagacaccag
     aggacgggtc tcccgggagg acgacaccag gttccccgtc gaccgacacg tctctcgtcc gtctgtggtc

 281 gctccaggac tgccaacctg gcaccgctgc ttggcgctgg gcgccctcta aaacaacaaa cttcgtgtct
     cgaggtcctg acggttggac cgtggcgacg aaccgcgacc cgcgggagat tttgttgttt gaagcacaga

 351 ctaatttggc acttcctgtt tatctttata ttatctcctg tccttgaagc tcacaggctc aatcaaggat
     gattaaaccg tgaaggacaa atagaaatat aatagaggac aggaacttcg agtgtccgag ttagttccta

 421 aatgcaaagg gccagaactt tggggactca gagatggaag aggcaagggt gccctctctg ccagggttct
     ttacgtttcc cggtcttgaa acccctgagt ctctaccttc tccgttccca cgggagagac ggtcccaaga

 491 aggctgcgcc cacaggcact gagcagaggt ctgaagtcca atgctccagg cactacagga ggctgagtct
     tccgacgcgg gtgtccgtga ctcgtctcca gacttcaggt tacgaggtcc gtgatgtcct ccgactcaga

 561 cctctgcacc tacacaacag gaagtcccac atgctgggtt ctagttgtcc caggttcata gatcaatgcc
     ggagacgtgg atgtgttgtc cttcagggtg tacgacccaa gatcaacagg gtccaagtat ctagttacgg

 631 acttttccc ctctacgaat cttggttttc tgaacaccac agaataggaa ggattcagca ggagaatccc
     tgaaaaaggg gagatgctta gaaccaaaag acttgtggtg tcttatcctt cctaagtcgt cctcttaggg

 701 ctagcttcac tgctgtctct ttctcatccg cacatggctt ggaatcactt cttaatgaat gacttaactc
     gatcgaagtg acgacagaga aagagtaggc gtgtaccgaa ccttagtgaa gaattactta ctgaattgag

 771 ctttcacagc ctttgagaaa tggatctaac tctgggtgct ttacctttt ttttttttt ttttttgag
     gaaagtgtcg gaaactcttt acctagattg agacccacga aatggaaaaa aaaaaaaaaa aaaaaactc

 841 acaaagtctc gctctgtggc ccaggctgga gtgcagtgtt gcgatctcag ttcactgcaa ccttcacttc
     tgtttcagag cgagacaccg ggtccgacct cacgtcacaa cgctagagtc aagtgacgtt ggaagtgaag

 911 ccaggttcaa acgattctcg tgcctcagcc tcccaagtag cttggactac aggcgtgtgc caccacaccc
     ggtccaagtt tgctaagagc acggagtcgg agggttcatc gaacctgatg tccgcacacg gtggtgtggg

 981 aatttttttt ttttttttt tgagacttag tcttgctctg tcacccagcc tggagggcag tggcacgatc
     ttaaaaaaaa aaaaaaaaaa actctgaatc agaacgagac agtgggtcgg acctcccgtc accgtgctag

1051 tcagttcatt gcaacctctg cctcccaggt tcaagcaatt cttctgcctc agcctcccaa gttgctggga
     agtcaagtaa cgttggagac ggagggtcca agttcgttaa gaagacggag tcggagggtt caacgaccct

1121 ttataggcac ccgccaccag acctggctaa tttttgtatt tcatagagat ggagtttcac cacgttggcc
     aatatccgtg ggcggtggtc tggaccgatt aaaaacataa agtatctcta cctcaaagtg gtgcaaccgg

1191 aggctggtct cgaactcctg acctccggtg atccactcat cttggcctct caaacagctg ggattacagg
     tccgaccaga gcttgaggac tggaggccac taggtgagta gaaccggaga gtttgtcgac cctaatgtcc

1261 catgagccac caccatgcca ggctttttttt ttttttttct gagacagggt ctcattctgt ctcccaggct
     gtactcggtg gtggtacggt ccgaaaaaaa aaaaaaaaga ctctgtccca gagtaagaca gagggtccga

1331 ggaatgcagt ggtgtgatca tagctcactg cagccttgac ctcctgggct caggtgatcc tcctacctca
     ccttacgtca ccacactagt atcgagtgac gtcggaactg gaggacccga gtccactagg aggatggagt

1401 gcctcccaag tagctgggac tacaggcacc ctcaccatgc ccaactaatt tttgtacttt ttgtagaaat
     cggagggttc atcgacccctg atgtccgtgg gagtggtacg ggttgattaa aaacatgaaa aacatcttta

1471 ggggtctggc tatgttgccc aagctggtct tgaatccctg ggcttaagca gtctgcccac tttggcctcc
     ccccagaccg atacaacggg ttcgaccaga acttagggac ccgaattcgt cagacgggtg aaaccggagg

1541 taaagtgcca ggattatagg tgtgagccac cgtggctggc tgcctcaggt tttgtttttt tttccagacg
     atttcacggt cctaatatcc acactcggtg gcaccgaccg acggagtcca aaacaaaaaa aaaggtctgc
```

```
1611 gagtctcact ctgtctgagt gagacccagg ctggagtgca gtggcatgat cttggctcac tgcaacctct
     ctcagagtga gacagactca ctctgggtcc gacctcacgt caccgtacta gaaccgagtg acgttggaga

1681 gcctcctggg ttcaagcgat tcttgtgcct cagcttccca agtagctggg actacaggcg cgcaccgcca
     cggaggaccc aagttcgcta agaacacgga gtcgaagggt tcatcgaccc tgatgtccgc gcgtggcggt

1751 tgcctggcta atttttgtat ttttggtaga gatgggtttt caccatgttg gccaggctgg tctcgaactc
     acggaccgat taaaaacata aaaaccatct ctacccaaaa gtggtacaac cggtccgacc agagcttgag

1821 ctgacctcaa gtgatccacc cacctcggct tcccaaagtg ctgggattac aggcataagc cactgcgcct
     gactggagtt cactaggtgg gtggagccga agggtttcac gaccctaatg tccgtattcg gtgacgcgga

1891 ggccttcagg ttcttgaatc catactctag gcacttgaaa gatgaagctt
     ccggaagtcc aagaacttag gtatgagatc cgtgaacttt ctacttcgaa
```

Figure 8: Complete sequence of pEpiGFP promoter clone #841. Maps to human Chromosome 17. Matches nucleotides 133874-135813 of Genebank Accession gi|18874231|gb|AC018521.8|[18874231]. Potentially important features noted within this region of chromosome 17 include two L2 repeats at position 133776-133894 and 133918-133975, and four Alu repeats at position 134689-134853 (AluSg/x), 134856-135146 (AluSx), 135157-135454 (AluJo) and 135463-135767 (AluSx).

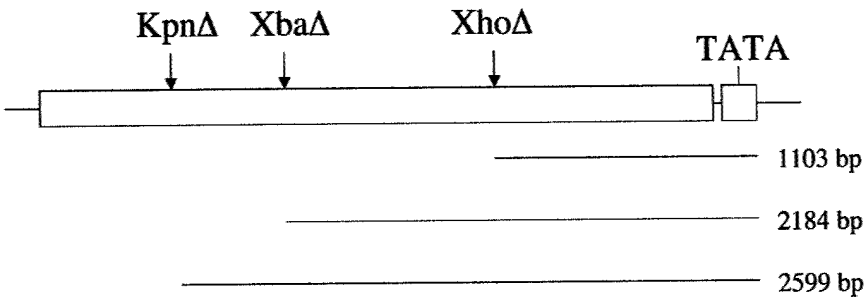
Table III: Activity of promoter clone 780 in various tumor cell lines

Tumor line	*Control*	*pTNF*	*pCMV*	*pRSV*	*p780*
K562	-	-	++	+	-
T24	-	+	+	+	+
293	-	-	+++	++	++
DU145	-	+	++	+	+
PC3	-	-/+	++	+	-

Promoter 780 and various control promoter constructs (CMV, RSV and TNF) were cloned upstream of a GFP indicator gene in an EBV-based episomal vector. Tumor cells were transduced by electroporation and GFP expression determined by FACS analysis.

FIGURE 9

(A)



(B)

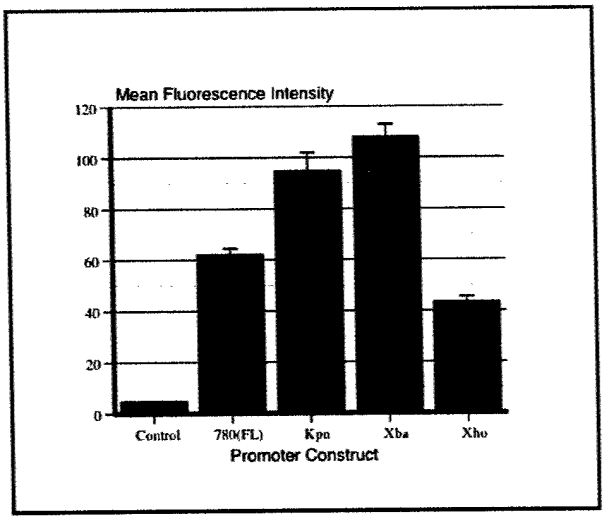


Figure 9: Deletional analysis of promoter clone 780. 5' deletions of the 780 promoter were prepared by restriction enzyme digestion and recloned upstream of the TATA box in pEpi(GFP) (A). Cells were transduced by electroporation and selected in G418. GFP expression was determined by FACS analysis (B).

FIGURE 10

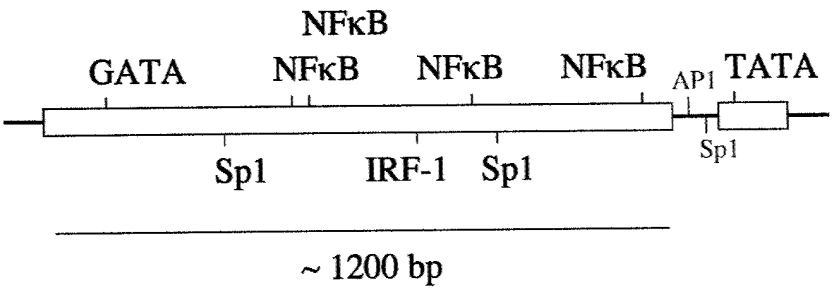


Figure 10: Human TNF-α promoter. The ~100 bp sequence adjacent to the TATA box containing an AP1 site and Sp1 site that appears to be responsible for the majority of the constitutive activity of element (see Figure 11) is indicated.

FIGURE 11

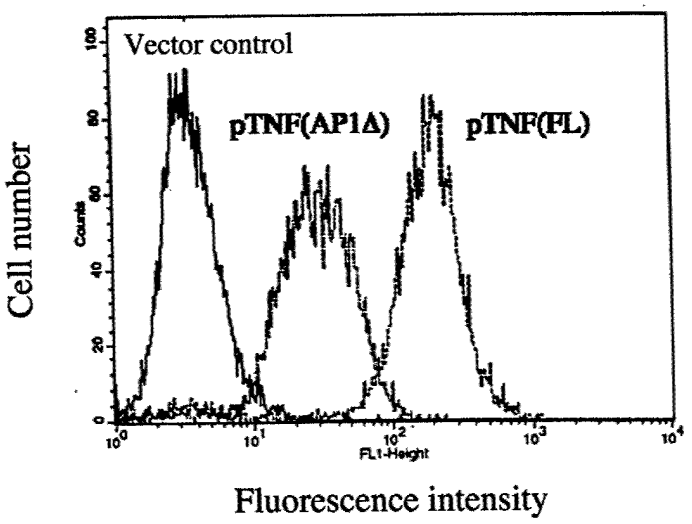


Figure 11: Activity of full length [pTNF(FL)] and AP1 deleted [pTNF(AP1D)] TNF promoter constructs in T24 cells. Promoter constructs were cloned upstream of a GFP indicator gene in an EBV-based episomal vector. Cells were transduced by electroporation and selected in G418. GFP expression was determined by FACS analysis. Removal of the AP1 site adjacent to the TATA box (see Figure 10) reduced promoter activity (as determined by mean fluorescence values) by 80-90% (note log scale).

FIGURE 12

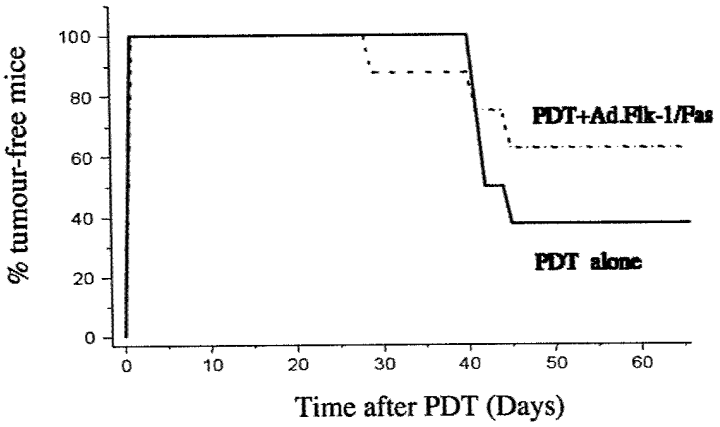


Figure 12: DU145 tumor xenografts (~5 mm in diameter) growing subcutaneously in SCID mice received a single peritumoral injection of $3.9x10^9$ PFU Ad.Flk-1/Fas. 24 hours later tumors were treated with PDT (mTHPC 0.1 mg/kg; 30 J/cm^2) and the impact of adenoviral-mediated gene transfer on tumor regrowth determined.